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MICROBIOLOGICAL, PHYSICAL-CHEMICAL, AMINO ACIDS AND MINERALS ANALYSIS OF POLLEN STORED IN HIVES OF *Melípona seminigra*, AMAZONAS, BRAZIL

Antonio BATISTA DA SILVA[1,*], Teresa ALARCÓN CASTILLO[2],
Maria Francisca SIMAS TEIXEIRA[3], Ana Lucia FIGUEIREDO PORTO[4]

[1] Federal University of Amazonas (UFAM). General Rodrigo Otávio Avenue, 6200, Coroado I District, CEP: 69067-005, Manaus Municipality, Amazon state, Brazil

[2] National Intercultural University of the Amazon (UNIA). San José de Tushmo highway km 0.63, Yarinacocha District, Coronel Portillo province, Ucayali region, Peru

[3] Federal University of Amazonas (UFAM). General Rodrigo Otávio Avenue, 6200, Coroado I District, CEP: 69067-005, Manaus Municipality, Amazon State, Brazil

[4] Rural Federal University of Pernambuco (UFRP). Dom Manoel de Medeiros Street, s/n, Dois Irmãos District, CEP: 52171-900, Recife Municipality, Pernambuco State, Brazil.

* e-mail: absilva@ufam.edu.br

ABSTRACT

Bee's pollen comes from the flowers of several species of plants. The grains are transported by worker bees to their nests (honeycombs) to be used as a source of nutrients. The objective of this study was to evaluate the microbiological control, the physical chemical composition and to analyze macro and micro minerals, as well as amino acids in dehydrated pollen hives of *Melipona seminigra*, in rainy periods (PLL) and dry periods (PS) in Amazonas, Brazil. The physical-chemical composition (proteins, lipids, carbohydrates, fibers and ashes) was performed by the AOAC methodology. In meliponícola pollen it was detected the presence of proteins (35,97 - 36,37 g/100g), lipids (22,37 - 29,66 g/100g), ashes (4,46 - 2,83 g/100g) and ratio carbon/nitrogen (8,94 - 8,28 g/100), also minerals like copper, iron, manganese, calcium, potassium, magnesium and phosphorus were present. In the pollen of the indigenous bees, both non-essential and essential amino acids were found. The results showed that the meliponícola pollen did not present any contamination for bacteria and fungi that comply with the Brazilian legislation. The physical-chemical analysis

confirmed that pollen has nutritional amounts that can be added to other ingredients, providing enrichment of fibers, minerals and proteins to foods at different ages of the population.

KEYWORDS: stingless bees, meliponiculture, Amazonia.

ANÁLISIS MICROBIOLÓGICO, FÍSICO-QUÍMICO, AMINOÁCIDOS Y MINERALES DEL POLEN ALMACENADO EN COLMENAS DE *Melípona seminigra*, AMAZONAS, BRASIL

RESUMEN

El polen de abeja proviene de las flores de varias especies de plantas. Los granos son transportados por abejas obreras hasta los nidos (panales) para ser usados como fuente de nutrientes. El objetivo de este estudio fue evaluar el control microbiológico, la composición físico química y analizar macro y micro minerales, así como aminoácidos en colmenas de polen deshidratado de *Melipona seminigra*, en épocas lluviosas (PLL) y secas (PS) en Amazonas, Brasil. La composición físico-química (proteínas, lípidos, carbohidratos, fibras y cenizas) se realizó mediante la metodología AOAC. En polen meliponícola se detectó la presencia de proteínas (35,97 - 36,37 g/100g), lípidos (22,37 - 29,66 g/100g), cenizas (4,46 - 2,83 g/100g) y la proporción de carbono/nitrógeno (8,94 - 8,28 g/100), también estuvieron presentes minerales como cobre, hierro, manganeso, calcio, potasio, magnesio y fósforo. En el polen de las abejas indígenas, fueron encontrados aminoácidos no esenciales y esenciales. Los resultados mostraron que el polen meliponícola no presentó ninguna contaminación por bacterias y hongos que cumplan con la legislación brasileña. El análisis físico-químico confirmó que el polen tiene cantidades nutricionales que se pueden agregar a otros ingredientes, proporcionando un enriquecimiento de fibras, minerales y proteínas a los alimentos en las diferentes edades de la población.

PALABRAS CLAVE: abejas sin aguijón, meliponicultura, Amazonía.

INTRODUCCIÓN

The rational breeding of stingless bees called meliponiculture is becoming a common and widespread practice in Brazil (Machado *et al*., 2019; Klein *et al*., 2020; Moreno, 2020). In the Amazon, there are many factors with potential for domestication and use of these bees known as yellow Jandaíra *Melipona* (Michmelia) *seminigra seminigra*, Friese, 1903 that besides pollinators of wild and cultivated species, are also used for honey, propolis and pollen production (Ribeiro *et al*., 2018; Silva *et al*., 2020; Souza *et al*., 2020). The pollen is mainly composed of proteins, lipids, sugars, fiber, minerals, amino acids and vitamins Camarena & Miranda (2017). It is a biological stimulant and human use has been for both purposes as for food and for medicinal purposes. It also has a content of essential nutrients for keeping bees, plus antioxidants and polyphenols (Ares *et al*., 2018; Végh *et al*., 2021). Animal studies have shown the use of pollen in the treatment of iron deficiency anemia (Rzepecka-Stojko *et al*., 2015; Visquert, 2015), increased calcification of bone components (Tomaszewska *et al*., 2020), inhibition of osteoclastic resorption (Haščík *et al*., 2017) with anti-inflammatory effect (Denisow & Denisow-Pietrzyk, 2016; Li *et al*., 2019), detoxifying properties of organochlorine pesticides (El-Ballal *et al*., 2019; Nassar *et al*., 2020), acts in mitigation of chemotherapy side effect (Kocot *et al*., 2018; Mărgăoan *et al*., 2019) and as an immunostimulant and anti-allergic agent (Khalifa *et al*., 2021). Also, increase apoptosis in ovarian cysts due to its phytoestrogenic properties (Naseri *et al*., 2021). Moreover, bee pollen polysaccharide from *Rosa rugosa* Thunb. (Rosaceae) promotes pancreatic β-cell proliferation and insulin secretion (Yang *et al*., 2021). In addition, pollen can be used to prevent some diseases that harm human health (Aylanc *et al*., 2021). Other studies using pollen have also demonstrated antifungal, antimicrobial, antiviral, hepatoprotective, anticancer and local analgesic action (Asoutis *et al*., 2020; Özkök *et al*., 2021), as well as polyunsaturated fatty acids, organic acids, some secondary metabolites such as phenolic acids, flavone aglycones and phenolamides (Qiang *et al*., 2018; Zhang *et al*., 2022). Also, vitamins such as β-carotene are commonly found in bee pollen (Salazar *et al*., 2020; Torres *et al*., 2020). For the other side were verified essential and non-essential amino acids (Thakur & Nanda, 2020; Ecem *et al*., 2021). Quality control of food intends to enhance good practice in hygiene procedures and food handling, establishing identity parameters and minimum requirements for the quality of bee pollen (ANVISA, 2001). In addition to fungi, mycotoxins and bacteria (Sinkevičienė *et al*., 2019; Jesus *et al*., 2021; Nuvoloni *et al*., 2021), inorganic contaminants are also present in the bee pollen, as is the case analyzed with dehydrated bee pollen samples from the Brazilian South, which obtained levels of Ba followed of V, Li, Cd and Pb (Sattler *et al*., 2016). Despite the nutritional importance of pollen, observed in several investigations all over the world, there is no scientific data on the nutritional potential of this product in Amazonian Meliponiculture, especially the native one from the lower Amazon, region where its consumption is restricted to its use in alternative treatment medicine for respiratory infections. This study was aimed at evaluating the microbiological control, physico-chemical composition, plus minerals and amino acids of pollen collected by *Melipona seminigra seminigra* from a community from lower Amazon, in the state of Amazonas, Brazil, during the rainy and dry periods.

METODOLOGY

The pollen collected by *M. seminigra* was acquired from meliponary located in Boa Vista do Ramos, belonging to the micro region of Parintins, State of Amazonas, Brazil (2° 58' 10,93'' S, 57° 35' 20,55" W), in the 2012 rainy and dry seasons (CNES/ Airbus, 2022). After collecting the pollen, it was dehydrated at 45 °C in an air circulation oven and we proceeded to the analysis for microbiological quality control, using Mac Conkey Agar, destined to the growth of gram-negative and lactose fermentation bacteria. For mycological control, Czapek Yeast Extract Agar (CYA), Sabouraud Agar and Malt Agar were used. The growth of microorganisms was monitored every 24 hours for five days in cultivations maintained at 25 °C, being all the samples processed in triplicate (Silva *et al*., 2017). The analysis was performed according to the methodology of the AOAC (1997) for moisture, total fat, total protein, carbohydrates and ashes. The macro and micro minerals were analyzed by atomic absorption spectroscopy (AAS) and 932 plus flame photometer (Malavolta *et al*., 1997). The nitrogen carbon ratio was analyzed according to the methodology by Pella (1990); Nelson & Sommers (1996); using the vario MAX CN elemental analyzer, at the National Institute de Research of the Amazonia - INPA and amino acids were detected by HPLC (White *et al*., 1986; Hagen *et al*., 1989). The water activity in the final product was measured with AquaLab apparatus and the analysis pH was performed using Micronal Potentiometer model B374.

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL COMPOSITION

The results of the physico-chemical composition of meliponicola pollen are exposed in **Table 1**. The pollen samples *M. seminigra seminigra* showed lower values of moisture content (8,09 g /100 g) however, had high amounts of protein (35,97 and 36,37 g / 100 g) and lipids (22,37 and 29,66 g / 100 g), while ash values (4,46 and 2,83 g / 100 g) during the rainy period (RP) and dry period (DP) were similar when compared to other studies (0,9 - 3,4 g / 100 g) (Albores-Flores *et al*., 2021). In addition, Góes (2019), working with *M. interrupta* in the Parintins municipality region in rainy and dry seasons, did not observe many variations with respect to humidity values of 23,1 and 23,2%; 22,85 and 23,19 g / 100 g of protein; 3,32 and 3,5 g / 100 g of lipids and 2,7 and 3,2 g / 100 g of ashes, respectively. Instead, the physical-chemical composition of bee pollen samples of *M. subnitida* in Alagoas, Northeast region of Brazil collected during rainy season, presented averages lower values of 7,8 g / 100 g for protein and 2 g / 100 g for lipids (Fernandes *et al*., 2018). Also, Figueredo *et al*. (2018), verified existing physical-chemical differences in pollens stored by different species of Meliponas distributed in Alagoas, Pernambuco and Bahia, Northeast of Brazil, being *M. escutellaris* which presented humidity values (50,05 %); protein (30,37 g / 100 g); lipids (5,99 g / 100 g) and ashes (4,21 g / 100 g). On the other hand, the results found in this investigation were similar to the experiments of Sarmento *et al*. (2016) who worked with *M. seminigra* and *M. interrupta* pollen in Manaus, Northwest region of Brazil, being that the physical chemical composition of *M. seminigra* pollen was more expressive in terms of values that reached 53,39 % of humidity; 37,63 g /100 g of proteins, 10,81 g /100 g of lipids and 4,03 g /100 g of ashes. In turn, in pollen collected by bees of the genus Melipona in Northeastern from the meliponaries of São Cristóvão, Maranhão and Bahia, *Gomes et al*. (2019), detected higher amounts of protein in *M. compressipes* (44,41 g / 100 g) and in *M. q. anthidioids* (39,1 g / 100 g) but

Table 1. Composition physico-chemical of pollen of *M. seminigra*.

Components	RP g / 100 g	DP g / 100g
Moisture	8,09 ± 0,82	8,39 ± 0,03
Protein	35,97 ± 0,85	36,37 ± 0,63
Lipids	22,37 ± 0,69	29,66 ± 0,68
Ashes	4,46 ± 0,05	2,83 ± 0,23
Fiber	2,17 ± 0,13	2,67 ± 0,11
Total Carbohydrates	26,42	20,57
Nitrogen	5,758	5,819
Energy (kcal)	471,18	449,25
pH	4,43	4,06
Aw	0,53	0,55
Soluble Solids (° Brix)	0,56	0,56
Carbon/Nitrogen Ratio	8,94	8,28

RP: Rainy Period; DP: Dry Period; Aw: Water Activity; pH: Hydrogen Potential; °Brix: Soluble Solids.

lower amounts of lipids (4,79 and 1,48 g / 100 g). The physico-chemical composition of pollen collected by *Apis mellifera* differs from meliponicola pollen. Thereby, in bee-pollen samples from Italy were obtained levels 28,42 g / 100 g of proteins, 2,83 g / 100 g of lipids and 2,85 g / 100 g of ashes (Gabriele *et al*., 2015). Similar data were obtained in collected pollen samples in South Korea, with protein levels of 26,5 g / 100 g, lipids of 7 g / 100 g and ashes of 5,3 g /100 g (Ghosh & Jung, 2017). Costa *et al*. (2017), in the state of Sergipe, Brazil obtained 21,91 g / 100 g of proteins, 9,23 g/100 g of lipids and 3,61 g / 100 g of ashes. As well as Gardana *et al*. (2018), who evaluated phytochemical composition in pollen from Spain, Italy and Colombia, reporting 21,6 g / 100 g of protein, 6 g / 100 g of lipids and 2,1 g / 100 g of ashes. In Portugal, Cardoso (2020) obtained 26,26 g / 100 g of protein, 2,20 g / 100 g of lipids and 2,38 g / 100 g of ashes, Thus, Lima (2016) observed that at the end of the rainy season, the pollen collected by the bees had a better protein value (33,18 g / 100 g) when compared to the other seasons of the year. Concluding that exists collect of pollen during the beginning of the rains, in the dry water transition period and in the rainy period.

MINERALS AND AMINO ACIDS

In the meliponicola pollen, it was detected the presence of micro minerals such as copper, iron and manganese as well as macro minerals such as calcium, potassium, magnesium and phosphorus **(Table 2),** finding iron and manganese in higher concentration during the DP. Macro minerals showed no significant difference between the periods evaluated. Worth mentioning that the amount of phosphorus and iron found in the Amazon meliponicola pollen was higher in 95 %. Unlike pollen of stingless bees from Parintins that expressed the highest concentrations of potassium, phosphorus, calcium and magnesium both in RP and DP, with the exception of iron and manganese whose amounts were lower than those found in this work (Góes, 2019). On the contrary, Kalaycıoğlu *et al*. (2017) obtained 0,49 g / 100 g

Table 2. Concentration of macro and micro minerals of pollen of *M. seminigra*

Microminerals			Macrominerals		
Acronym	RP g / 100 g	DP g / 100 g	Acronym	RP g / 100 g	DP g / 100 g
Cu	7,78	18,25	Ca	3,02	3,29
Fe	61,18	81,14	K	0,09	0,19
Mn	ND	33,21	Mg	0,18	0,17
			P	5,79	7,09

Cu: Copper; Fe: Iron; Manganese: Mn; Calcium: Ca; K: Potassium; Mg: Magnesium; Phosphorus: P; ND: Not detected; Rainy Season: RP; Dry Season: DP.

Table 3. Concentration of amino acids of pollen of *M. seminigra*.

Amino Acids					
Acronym	RP g / 100 g	DP g / 100 g	Acronym	RP g / 100 g	DP g / 100 g
Essential amino acids			**Nonessential amino acids**		
Thr	1,24	1,33	Gly	1,54	1,67
Met	0,73	0,72	Asp	3,07	3,10
Arg	1,83	1,93	Glu	3,58	3,84
Val	1,71	1,11	Ala	1,88	2,02
Phe	1,57	1,65	Pro	1,80	1,94
Lys	2,42	2,34	Ser	1,56	1,66
His	0,66	0,63	Cys	0,19	0,18
Ile	1,49	1,60	Tyr	1,05	1,94
Leu	2,62	2,77			
TOTAL	28,94	30,43	TOTAL	14,67	16,35

Alanine: Ala; Ác.Aspártico: Asp; Ác.Glutámico: Glu Arginine: Arg; Cysteine: Cys; Phenylalanine: Phe; Glycine: Gly; Histidine: His; Isoleucine: Ile; Leucine: Leu; Lysine: Lys; Methionine: Met; Proline: Pro; Serine: Ser; Threonine: Thr; Tyrosine: Tyr; Valine: Val; Rainy Season: RP; Dry Season: DP.

(K); but 0,24 g / 100 g (Ca); 0,11 g / 100 g (Mg); 0,02 g / 100 g (Fe); 0,004 g / 100 g (Mn) and 0,001 g / 100 g (Cu), likewise Spulber *et al*. (2018), reached concentrations of 0,43 g / 100 g (K); 0,07 g / 100 g (Mg) and 0,43 g / 100 g (Ca) and only high concentrations of 0,77 g/ 100 g (K) and 0,39 g / 100 g (Mg) determined in the experiments of Taha & Al-Kahtani (2020). Costa *et al*. (2019), detected 0,74 g / 100 g (K) and 0,69 g / 100 g (P), 0,37 g / 100 g (Ca); 0,17 g / 100 g (Mg); in the states of Sergipe, Piaui and Mato Grosso, too. In addition to Zafeiraki *et al*. (2022), who analyzed bee pollen samples from Greece and reported higher concentration of K (4,04 g / 100 g), followed by P (0,483 g / 100 g), Ca (0,165 g / 100 g), Mn (0,61 g / 100 g) and Fe (0,149 g / 100 g). In the pollen of these indigenous bees, both non-essential and essential amino acids essentials are found **(Table 3).** Among the nonessential amino acids, aspartic acid content (3,07 and 3,10 g / 100 g) and glutamic acid (3,58 and 3,84 g / 100 g) were the highest. On the other hand, within the essential amino acids, it was prevalent leucine [2,62 (RP) - 2,77 (DP) g / 100 g] and lysine [2,42 (RP)

- 2,34 (DP) g / 100 g]. Nevertheless, the amounts of amino acids found in the present investigation were surpassed by Gomes *et al*. (2019), who detected high concentrations of asparagine (11 to 18 g / 100 g); followed by glutamic acid (11 to 13 g / 100 g); proline (8 to 13 g / 100 g) and leucine (8 to 10 g / 100 g) in pollen collected by most of the Meliponas species studied. In other studies, bee pollen from Botucatu, Brazil, it was detected levels of glutamic acid in summer (1,82 g / 100 g) and in winter (1,89 g / 100 g) but the levels of proline during the summer (2,12 g / 100 g) and (2,36 g / 100 g) in winter, were the highest (Negrão & Orsi, 2018). For Taha *et al*. (2019), five amino acids prevailed in the bee pollens tested: glutamic acid (1,84 g / 100 g), glycine (1,76 g / 100 g), aspartic acid (1,64 g / 100 g), alanine (1,34 g / 100 g) and leucine (1,2 g / 100 g). Finally, Al-Kahtani *et al*. *(*2020), determined the composition of bee pollen in eastern Saudi Arabia during 4 seasons; showing the highest amounts of glutamic acid (1,69 g / 100 g); glycine (1,67 g / 100 g); aspartic acid (1,62 g / 100 g); leucine content (1,29 g / 100 g); valine (1,03 g / 100 g) isoleucine (0,6 g / 100 g); tyrosine and cysteine (0,2 g / 100 g) in samples collected during spring.

EVALUATING THE MICROBIOLOGICAL CONTROL

The results of the microbiological quality control of dehydrated pollen meliponicola *M. seminigra seminigra* showed no contamination by total coliforms, thermotolerant and fungi according to health legislation (ANVISA, 2001).

CONCLUSIONS

In accordance with the results obtained, it can be concluded that in the analysis of quality control of dehydrated pollen there was no contamination by bacteria or fungi responding to the demands of the Brazilian legislation. The physico-chemical analysis of pollen exhibited nutritional amounts that can be incorporated into other ingredients providing enrichment fiber, minerals and protein to food for different age groups of the population, without compromising nutrient consumption and confirming the great potential of this product from the Amazon meliponiculture as a dietary supplement in the human diet.

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